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***Nocardia kroppenstedtii* sp. nov., a novel actinomycete isolated from a lung  
transplant patient with a pulmonary infection**

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The GenBank accession number for the 16S rRNA gene sequence of strain N1286<sup>T</sup> is  
DQ157924.

## Abstract

An actinomycete, strain N1286<sup>T</sup>, isolated from a lung transplant patient with a pulmonary infection, was provisionally assigned to the genus *Nocardia*. The strain had chemotaxonomic and morphological properties typical of members of the genus *Nocardia* and formed a distinct phyletic line in the *Nocardia* 16S rRNA gene tree. It was most closely related to *Nocardia farcinica* DSM 43665<sup>T</sup> (99.8% gene similarity) but was distinguished from the latter by a low level of DNA:DNA relatedness. These strains were also distinguished by a broad range of phenotypic properties. On the basis of these data, it is proposed that isolate N1286<sup>T</sup> (=DSM 45810<sup>T</sup> = NCTC 13617<sup>T</sup>) should be classified as the type strain of a new *Nocardia* species for which the name *Nocardia kroppenstedtii* is proposed.

Improvements in the classification of the genus *Nocardia* due to the application of polyphasic taxonomy provide a sound framework for the recognition of additional species (Goodfellow & Maldonado, 2012). At the time of writing, the genus encompasses 85 validly published species (<http://www.bacterio.net/n/nocardia.html>), including the recently described *Nocardia grenadensis* (Kämpfer *et al.*, 2012), *Nocardia rhamnosiphila* (Everest *et al.*, 2011), *Nocardia goodfellowii* and *Nocardia thraciensis* (Sazak *et al.*, 2012). Nocardiae form a clade within the evolutionary radiation occupied by mycolic acid-containing actinomycetes, that is, microorganisms belonging to genera assigned to the order *Corynebacteriales* (Goodfellow & Jones, 2012). Most recently described *Nocardia* species are associated with human infections (Brown-Elliott *et al.*, 2006; Goodfellow & Maldonado, 2012), as exemplified by *Nocardia mikamii* (Jannat-Khah *et al.*, 2010) and *Nocardia niwae* (Moser *et al.*, 2011). Here we describe the results of phenotypic and phylogenetic

analyses of another strain isolated from clinical material and show that it represents a new *Nocardia* species.

Strain N1286<sup>T</sup> was isolated from bronchial lavage cultured on chocolate agar incubated at 37°C in 5% CO<sub>2</sub> for 2 days. The organism was maintained on glucose-yeast extract agar (GYEA; Gordon & Mihm, 1962) at room temperature and as glycerol suspensions (20%, v/v) at -20°C, as were *Nocardia asteroides* DSM 43757<sup>T</sup> and *Nocardia farcinica* DSM 43665<sup>T</sup>. Biomass of all strains analysed, for the chemotaxonomic and molecular systematic studies was grown in shake flasks of GYE broth for 5 days at 28°C, checked for purity and harvested by centrifugation. Cells for the chemosystematic analyses were washed twice in distilled water and freeze-dried; those for the molecular systematic work were washed in NaCl/EDTA buffer (0.1M EDTA, 0.1M NaCl, pH 8.0) and stored at -20°C until required.

The phylogenetic position of isolate N1286<sup>T</sup> was determined by 16S rRNA gene sequence analysis. Chromosomal DNA was isolated, PCR fragments amplified and direct sequencing of the purified products carried out after Kim *et al.*, (1998). The almost complete 16S rRNA gene sequence (1544 nucleotides [nt]) was aligned manually against corresponding sequences of genera classified in the order *Corynebacteriales*, retrieved from the DDBJ/EMBL/GenBank databases, using the pairwise alignment option and 16S rRNA secondary structural information held in the MEGA5 program (Tamura *et al.*, 2011). Phylogenetic trees were inferred using the maximum-parsimony (Kluge & Farris, 1969), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the MEGA5 software. The Jukes and Cantor (1969) model was used to generate an

evolutionary distance matrix for the neighbour-joining algorithm. Topologies of the resultant unrooted trees were evaluated by bootstrap analysis of the neighbour-joining method (Felsenstein, 1985) based upon 1000 replicates using MEGA 5 software.

It can be seen from Figures 1 and S2, that strain N1286<sup>T</sup> formed a distinct subclade in the 16S rRNA *Nocardia* gene tree together with the type strain of *N. farcinica*, an association supported by all of the tree-making algorithms and by a 99% bootstrap value in the neighbour-joining analysis. The strains shared a 16S rRNA gene similarity of 99.8%, a value that corresponded to 3 nt differences at 1544 locations. The two strains were associated with the type strains of *Nocardia higoensis* and *Nocardia shimofuensis*, as shown in Figure 1; strain N1286<sup>T</sup> shared 16S rRNA similarities of 98.9% with the *N. higoensis* and *N. shimofuensis* strains, a value equivalent to 17 nt differences.

Strain N1268<sup>T</sup> was examined for key chemotaxonomic markers considered to be characteristic of *Nocardia* strains using *N. asteroides* DSM 43757<sup>T</sup> as control. Standard procedures were used to determine the diagnostic isomers of diaminopimelic acid (A<sub>2</sub>pm; Stanek & Roberts, 1974), cellular fatty acids (Sutcliffe, 2000), isoprenoid quinones (Collins, 1994), muramic acid type (Uchida *et al.*, 1999), mycolic acids (Minnikin *et al.*, 1975), polar lipids (Minnikin *et al.*, 1984) and whole-organism sugars (Hasegawa *et al.*, 1983). The organism contained *meso*-A<sub>2</sub>pm, arabinose and galactose, in whole-organism hydrolysates (wall chemotype IV sensu, Lechevalier & Lechevalier, 1970); N-glycolyl muramic acid; hexahydrogenated menaquinone with eight isoprene units where the two end units were cyclized (MK- 8 [H<sub>4</sub>], ω cyclo) as the sole isoprenologue; major proportions of straight chain saturated, unsaturated and

tuberculostearic acids (fatty acid type 1b *sensu*, Kroppenstedt, 1985), diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol phosphatidylinositolmannosides as major polar lipids (Fig S1.); and mycolic acids that co-migrated with those from the type strain of *N. asteroides*. This chemotaxonomic profile is consistent with the classification of isolate N1268<sup>T</sup> in the genus *Nocardia* (Goodfellow & Maldonado, 2012).

DNA:DNA relatedness values ( $\Delta T_m$ ) were determined, in triplicate, between isolate N1286<sup>T</sup> and *N. farcinica* DSM 43665<sup>T</sup> using the fluorimetric method described by Gonzalez and Sait-Jimenez (2005); the optimum temperatures for reassociation ( $T_{or}$ ) were calculated using the equation  $T_{or} = 0.51 (\%GC) + 47$ . The melting temperatures ( $T_m$ ) at which 50% of the initial double-stranded DNA molecules denatured into single-stranded DNA for isolate N1286<sup>T</sup> g DNA and isolate N1286<sup>T</sup> / *N. farcinica* hybrid DNA preparations were compared and the differences ( $\Delta T_m$ ) calculated. The %GC was 80.2%, the mean  $\Delta T_m$  between isolate N1286<sup>T</sup> g DNA and isolate N1286<sup>T</sup> / *N. farcinica* hybrid DNA was  $9.6 \pm 1.2^\circ\text{C}$ , a value which represents a DNA:DNA relatedness value of  $44 \pm 4\%$  (Gonzalez & Saiz-Jimenez, 2005).

Isolate N1286<sup>T</sup> and the type strain of *N. farcinica*, were examined for a range of phenotypic properties using well established media known to be of value in nocardial systematics (Andrews, 2001; Goodfellow, 1971; Goodfellow & Maldonado, 2012; Isik *et al.*, 1999). A number of differential characteristics separated the two strains; isolate N1286<sup>T</sup>, unlike the *N. farcinica* strain, grew at 37°C, did not produce aerial mycelium, degraded starch, hydrolysed aesculin and arbutin; grew on *meso*-inositol and methyl- $\alpha$ -D-glucopyranoside as a sole carbon source (1% w/v) and was not

inhibited by bacitracin (10 units). Similarly, *N. farcinica* DSM 43665<sup>T</sup>, unlike the isolate, degraded DNA, and RNA; reduced nitrate, and grew on dulcitol and *i*-erythritol (1% w/v) and on sodium benzoate, oxalic acid and pimelic acid (0.1% w/v) as sole carbon sources and in the presence of fusidic acid (10 µg).

It can be concluded that isolate N1286<sup>T</sup> forms a distinct phyletic line in the *Nocardia* 16S rRNA gene tree and can be distinguished readily from *N. farcinica* DSM 43665<sup>T</sup>, its nearest phylogenetic neighbour, using a combination of phenotypic features. Consequently, it is proposed that isolate N1286<sup>T</sup> should be recognised as a new species, *Nocardia kroppenstedtii*.

#### **Description of *Nocardia kroppenstedtii* sp. nov.**

*Nocardia kroppenstedtii* (krop. pen. sted'ti.i. N.L. n. *kroppenstedtii*, of Kroppenstedt to honour Reiner Kroppenstedt, a German microbiologist, for his many contributions to actinobacterial systematics).

Aerobic, Gram-positive, nonmotile, nonsporeforming, partially-acid alcohol fast, catalase-positive, actinomycete which forms a mycelium that fragments into rods and cocci. Irregular, wrinkled, matt, pale orange yellow pigmented colonies are formed on modified Bennett's agar after 5 days growth at 30°C. Growth occurs at pH 6.0-10.0, from 25°C to 37°C and optimally ~ 28°C. Uric acid is not degraded. D-arabitol, arbutin, D-fucose, glycerol and D-ribose (1%, w/v), *n*-propanol (1%, v/v) and γ-hydroxybutyric acid, sodium fumarate, sodium-DL-malate and sodium suberate (0.1%, w/v) are used as sole carbon sources. Growth occurs in the presence of filter paper discs soaked in bacitracin (10 units), cephalixin (30 µg), clindamycin

hydrochloride (2 µg), colistin (25 µg), cotrimoxazole (25 µg), erythromycin (5 µg), nalidixic acid (30 µg), novobiocin (5 µg), penicillin (1 µg) and tetracycline hydrochloride (10 µg), but not in the presence of discs soaked in ciprofloxacin (1 µg) and fusidic acid (10 µg). Additional phenotypic properties are cited in the text. The major cellular fatty acid components are hexadecanoic (C16:0; 30.8 %), monosaturated hexadecanoic (C16:1; 18.6 %), octadecanoic (C18:0; 7.2 %), monosaturated octadecanoic (C18:1; 6.4 %), tuberculostearic acid (TSA<sub>18</sub>; 30.2 %) and eicosanoic (C 20:0; 5.2 %). Additional chemotaxonomic properties are also typical of nocardiae.

The type strain, N1286<sup>T</sup> (=DSM 45810 = NCTC 13617<sup>T</sup>), was isolated from a lung transplant patient with a pulmonary infection. The species description is based on a single strain and hence serves as a description of the type strain.

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## References

- Andrews, J. M. (2001).** BSAC standardized disc susceptibility testing method. *J Antimicrob Chemother* **48 Suppl 1**, 43-57.
- Brown-Elliott, B. A., Brown, J. M., Conville, P. S. & Wallace, R. J., Jr. (2006).** Clinical and laboratory features of the *Nocardia* spp. based on current molecular taxonomy. *Clin Microbiol Rev* **19**, 259-282.
- Collins, M. D. (1994).** Isoprenoid quinones. In *Chemical Methods in Prokaryotic Systematics*, pp. 265-309. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: John Wiley & Sons.



- Everest, G. J., Cook, A. E., le Roes-Hill, M. & Meyers, P. R. (2011). *Nocardia rhamnosiphila* sp. nov., isolated from soil. *Syst Appl Microbiol* **34**, 508-512.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368-376.
- Felsenstein, J. (1985). Confidence-limits on phylogenies - an approach using the bootstrap. *Evolution* **39**, 783-791.
- Gonzalez, J. M. & Saiz-Jimenez, C. (2005). A simple fluorimetric method for the estimation of DNA-DNA relatedness between closely related microorganisms by thermal denaturation temperatures. *Extremophiles* **9**, 75-79.
- Goodfellow, M. (1971). Numerical taxonomy of some nocardioform bacteria. *J Gen Microbiol* **69**, 33-80.
- Goodfellow, M. & Maldonado, L. A. (2012). Genus I. *Nocardia* Trevisan 1889<sup>AL</sup>. In *Bergey's Manual of Systematic Bacteriology*, 2<sup>nd</sup> edn, vol. 5, pp. 376-419. Edited by M. Goodfellow, P. Kämpfer, H.-J. Busse, M. E. Trujillo, K.-I. Suzuki, W. Ludwig & W. B. Whitman. New York: Springer.
- Goodfellow, M. & Jones, A. L. (2012). Order V. *Corynebacteriales* ord. nov. In *Bergey's Manual of Systematic Bacteriology*, 2<sup>nd</sup> edn, vol. 5, pp. 232-243. Edited by M. Goodfellow, P. Kämpfer, H.-J. Busse, M. Trujillo, K.-I. Suzuki, W. Ludwig & W. Whitman. New York: Springer.
- Gordon, R. E. & Mihm, J. M. (1962). Identification of *Nocardia caviae* (Erikson) nov. comb. *Ann N Y Acad Sci* **98**, 628-636.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319-322.
- Isik, K., Chun, J., Hah, Y. C. & Goodfellow, M. (1999). *Nocardia salmonicida* nom. rev., a fish pathogen. *Int J Syst Bacteriol* **49** Pt 2, 833-837.
- Jannat-Khah, D., Kroppenstedt, R. M., Klenk, H. P., Sproer, C., Schumann, P., Lasker, B. A., Steigerwalt, A. G., Hinrikson, H. P. & Brown, J. M. (2010). *Nocardia mikamii* sp. nov., isolated from human pulmonary infections in the USA. *Int J Syst Evol Microbiol* **60**, 2272-2276.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism* vol. 3, pp. 21-132. Edited by H. N. Munro. New York: Academic Press.
- Kämpfer, P., Lodders, N., Grun-Wollny, I., Martin, K. & Busse, H. J. (2012). *Nocardia grenadensis* sp. nov., isolated from sand of the Caribbean Sea. *Int J Syst Evol Microbiol* **62**, 693-697.
- Kim, S. B., Falconer, C., Williams, E. & Goodfellow, M. (1998). *Streptomyces thermocarboxydovorans* sp. nov. and *Streptomyces thermocarboxyodus* sp. nov., two moderately thermophilic carboxydotrophic species from soil. *Int J Syst Bacteriol* **48**, 59-68.
- Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1-32.
- Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*, pp. 173-199. Edited by M. Goodfellow & D. E. Minnikin. London.: Academic Press.
- Lechevalier, M. P. & Lechevalier, H. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435-443.
- Minnikin, D. E., Alshamaony, L. & Goodfellow, M. (1975). Differentiation of *Mycobacterium*, *Nocardia*, and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. *J Gen Microbiol* **88**, 200-204.

- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984).** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233-241.
- Moser, B. D., Klenk, H. P., Schumann, P., Potter, G., Lasker, B. A., Steigerwalt, A. G., Hinrikson, H. P. & Brown, J. M. (2011).** *Nocardia niwae* sp. nov., isolated from human pulmonary sources. *Int J Syst Evol Microbiol* **61**, 438-442.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406-425.
- Sazak, A., Sahin, N. & Camas, M. (2012).** *Nocardia goodfellowii* sp. nov. and *Nocardia thraciensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **62**, 1228-1234.
- Staneck, J. L. & Roberts, G. D. (1974).** Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226-231.
- Sutcliffe, I. C. (2000).** Characterisation of a lipomannan lipoglycan from the mycolic acid containing actinomycete *Dietzia maris*. *Antonie van Leeuwenhoek* **78**, 195-201.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011).** MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731-2739.
- Uchida, K., Kudo, T., Suzuki, K. I. & Nakase, T. (1999).** A new rapid method of glycolate test by diethyl ether extraction, which is applicable to a small amount of bacterial cells of less than one milligram. *J Gen Appl Microbiol* **45**, 49-56.

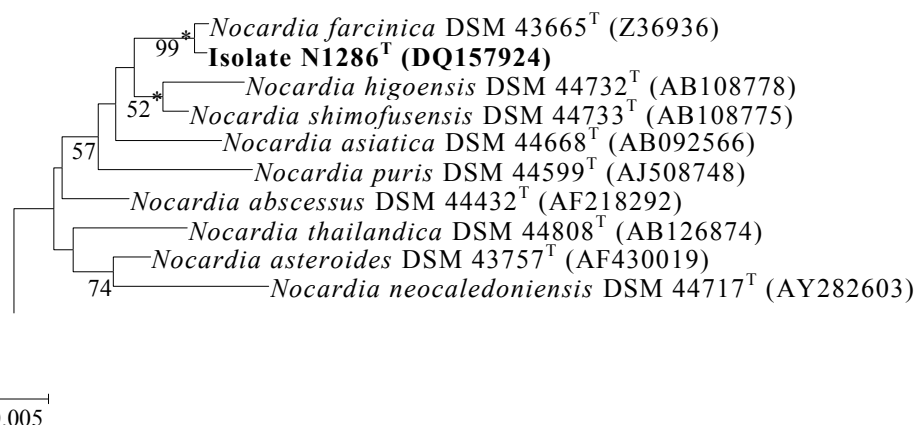


Fig.1. A section of the neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the position of strain N1286<sup>T</sup> relative to its nearest neighbours. Asterisks indicate branches of the tree that were also found with the maximum-likelihood and maximum-parsimony tree-making algorithms; L and M indicate branches found using the maximum-likelihood and maximum-parsimony methods, respectively. The numbers at the nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 re-sampled datasets; only values above 50% are given. The scale bar indicates 0.005 substitutions per nucleotide position. <sup>T</sup>, type strain.

Supplementary figures

Fig. S1: Polar lipid composition of strain N1286<sup>T</sup>. The polar lipids were identified as follows: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositolmannosides.

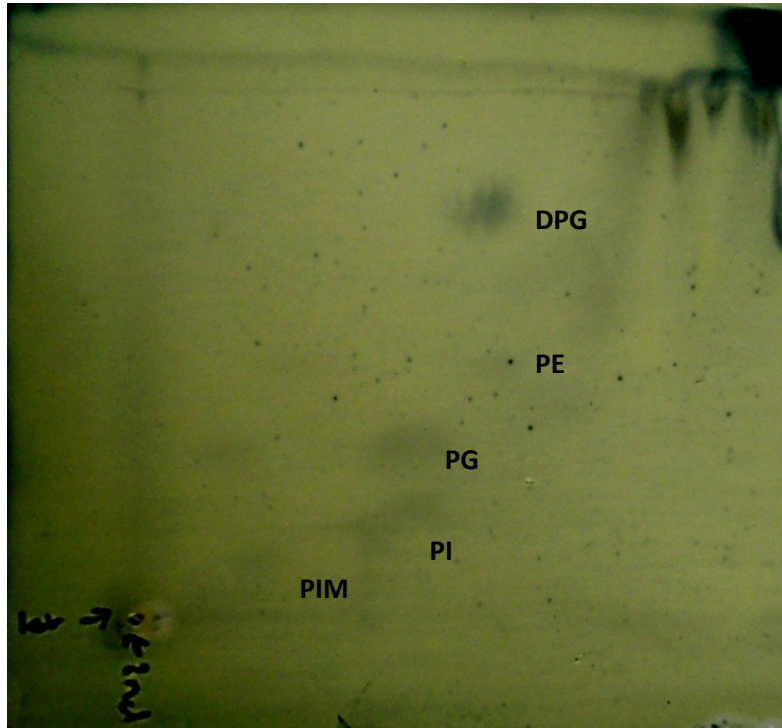


Fig. S2. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the position of strain N1286<sup>T</sup>. Asterisks indicate branches of the tree that were also found with the maximum-likelihood and maximum-parsimony tree-making algorithms; L and M indicate branches found using the maximum-likelihood and maximum-parsimony methods, respectively. The numbers at the nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 re-sampled datasets; only values above 50% are given. The scale bar indicates 10 substitutions per nucleotide position. <sup>T</sup>, type strain.

